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Synthesis and study of arginine -containing citrates

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Abstract

Different-ligand chelate compounds with a general formula $Me(Arg)_n \cdot HL \cdot mH_2O$, where Me=Co, Cu, Cr; n=1÷2; m=0÷4, HL – citrate-ion, have been synthesized. The synthesized compounds have been studied using a number of physical and chemical methods. The composition has been established via trace element analysis, while the individuality – by the methods of melting temperature measurement and X-Ray-diffractometric study. Using the method of conductometric study there have been measured chelate dissociation constants and determination coefficients. Qualitative solubility of chelates in different solvents has been determined. In order to establish the biological activity of the synthesized chelate compounds, an effect of premixes prepared on the basis of chelate compounds, on some hematological indicators of rabbits' blood has been studied.

Keywords: Different-Ligand; Citrate; Arginine; Rabbit; Blood; Hematological Parameter

1. Introduction

Microelements' deficiency is one of the main reasons stipulating low quantitative and qualitative indicators of the agricultural production in organisms of birds and animals. Crucial role in solution of this problem belongs to provision of the living organisms with optimum quantitative and qualitative ratio of microelements being in chelate form. This circumstance can be explained by the fact that the digestibility of microelements being in inorganic form by living organisms roughly equals to 10-17%, at that a deterioration of ecological state takes place. The digestibility of microelements being in chelate form by the organisms roughly equals to 60-70% [1-5]. Amino acids, vitamins and oxy-acids, along with essential microelements the most frequently play the role of chelate-forming ligand, that is reasoned by their special role and importance for living organisms.

In these recent times, interest in different-ligand chelate compounds has been especially heightened. Mostly it refers to such chelates, which contain organic ligands with different chemical and physical properties and biological activity [6-10.]. This interest is explained by the fact that in such case takes place the change of properties of reacting organic substances and microelements entering into composition of chelates. They acquire new, biologically active properties, which were not peculiar separately for ligands and microelements being in free state. For instance, microelements' toxicity drastically decreases and they acquire a skill to catalyze different biochemical processes etc. Taking a number of other factors into account, it is possible to generate new-generation coenzyme preparations and biological catalysts, new medications and biologically active additives [6-10].

Continuing the synthesis and study of this type of compounds [11], we have selected amino acid arginine of basic nature, and tri-basic oxy-acid citric acid as a subject of research. We have established the synthesis conditions and synthesized

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arginine-containing cobalt, copper and chromium citrates. Study of physical-chemical and biological activity of the synthesized compounds has been conducted as well.

Amino acid arginine is a nitrogen oxide donor, that promotes blood-vessel system relaxation and elasticity. This circumstance is of great importance for the treatment of a number of diseases (cardiovascular system, brain, immune and nervous system diseases, atherosclerosis, genital system disorders etc.). Arginine is critically important for poultry feeding, since it promotes bird growth, improvement of immunity and intestine health, and is a source of protein synthesis, hormone growth and nitrogen oxide (NO). Arginine deficiency causes poultry growth inhibition, feathering problems, bone deformation and decrease in productivity. It is vitally important for broiler's nutrition. Taking these factors into account, and according to literary sources, it is successfully used in poultry feeding in the composition of combined feed premixes [12-15].

Tri-basic oxy-acid – citric acid selected as an acid ligand is an important product of metabolism in living organisms. Participating in glyoxylate cycles of tri-basic acids, it is a main link of biochemical reaction system of cell respiration. Addition to poultry food ration of coordination (chelate) compounds of homo- and heteronuclear citrates obtained on the basis of citric acid promotes bird productivity increase, digestibility improvement, poultry meat and bone tissue quality increase, and enzyme activation [16-18].

2. Materials and used methods

In the synthesis of chelating compounds, cobalt acetate, copper acetate, chromium acetate, oxoacid - citric acid and amino acid - arginine were used. The above-mentioned reagents of the Sigma-Aldrich brand were purchased.

- Trace element analysis – for establishment of chelate compound compositions;
- Melting temperature determination and x-ray-diffractometric study – for establishment of chelates' individuality;
- Solubility – for study of compounds' qualitative solubility in different solvents;
- Conductometric study – for determination of dissociation constant of solutions containing chelate compounds;
- Determination of some blood hematological parameters – for study of rabbits' physiological state.

3. Results and Discussion

We have continued the synthesis of arginin-containing citrates [11], with a general formula

$Me(Arg)_n \cdot HL \cdot mH_2O$, aimed to synthesis of chelate compounds (where Me=Co, Cu, Cr; n=1÷2; HL^{-2}- citrate-ion, m=0÷4). Mixtures of metal acetates, arginine and citric acid in the molar ratio of 1:1:2 and 1:2:2 undergo dissolution under conditions of warming in minimal volume of water and vigorous stirring. Obtained real solutions are hold in the water steam bath, then the obtained compounds are treated with water in order to remove acetic acid residuals. Ultimately, the solutions are rinsed with small amount of water, ether and are dried at ambient temperature. Citrate compositions have been established via trace element analysis. There has been determined the qualitative solubility in different solvents, according to which chelates are distinguished by high water solubility, and by poor solubility in organic solvents (Table 1).

Table 1 Some physico-chemical characteristics of metal citrates containing arginine

#	The formula of the Compound	Mol Mass	Melting t 0_C	Humidity B (%)	Solubility				Conductometric Survey Results	
					Water	Alcohol	Acetone	DMSO *	pKa	R^2
1	$[CoArg]HL \cdot 6H_2O$	531.16	75	1.49	+	-	-	-	4.52	0.82
2	$[CoArg_2]HL$	597.33	71	1.97	+	-	-	-	3.57	0.79
3	$[CuArg]HL \cdot 6H_2O$	535.75	80	0.46	+	-	-	-	4.17	0.78

4	$[CuArg_2]HL\cdot 2H_2O$	638,.07	74	0.95	+	-	-	-	4.16	0.3
5	$[GrArg]HL\cdot 4H_2O$	487.67	110	0.65	+	-	-	-	4.03	0.73
6	$[GrArg_2]L\cdot 4H_2O$	662.15	105	0.89	+	-	-	-	4.60	0.79

+ Soluble, -Insoluble, DMSO- Dimethylsulfoxide

Through melting temperature determination, it has been established that they melt within the (71-110) temperature range. Except for melting temperature determination, the individuality of these compounds: $Co\cdot Arg\cdot HL\cdot 2H_2O$ (1), $[Cu(Arg)HL]\cdot 6H_2O$ (2), $Cr\cdot Arg\cdot L\cdot 4H_2O$ (3) has been established via the X-Ray-diffractographic method as well. Study of individuality and purity degree of synthesized compounds has been conducted with the use of ДРОН-4.07 at $Cu_{k\alpha}$(λ=0.15418бд) irradiation. During exposition, samples have been rotated in their eigenplane by means of special device – ГП-13. Diffractograms of the initial reacting compounds have been made for comparison, as well (Fig. 1-3).

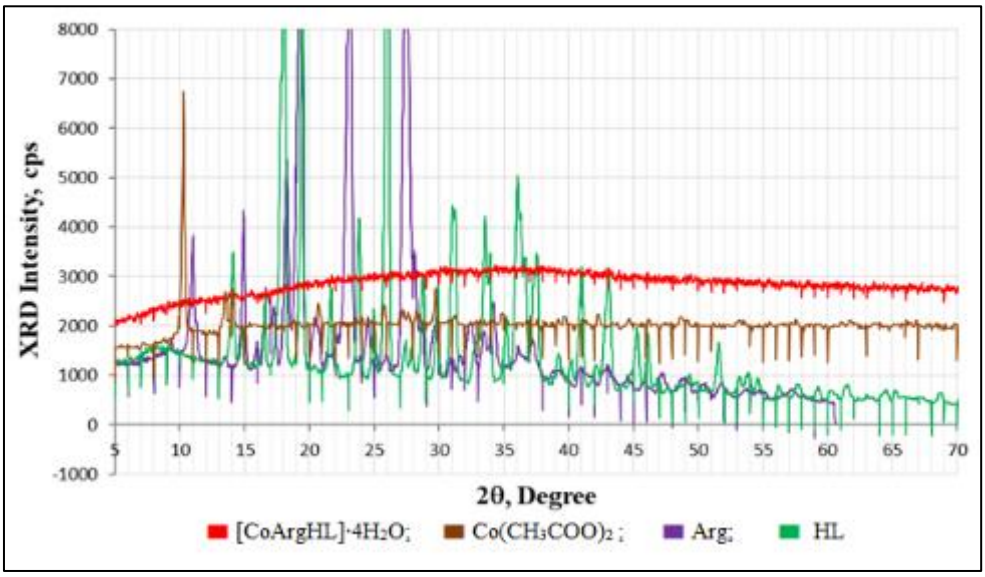

Figure 1 Diffractogram of the compound $CoArgHL]\cdot 4H_2O$

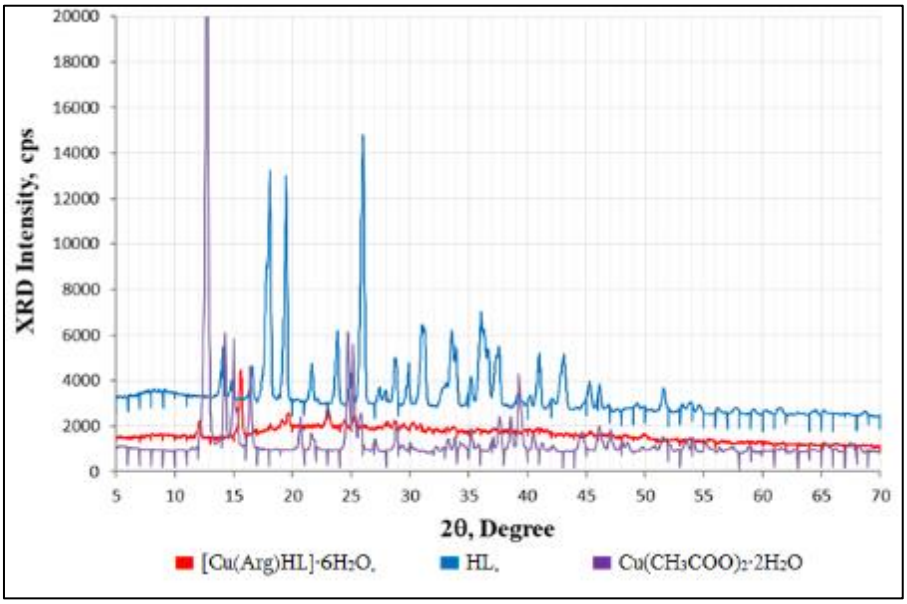

Figure 2 Diffractogram of the compound $[Cu(Arg)HL]\cdot 6H_2O$

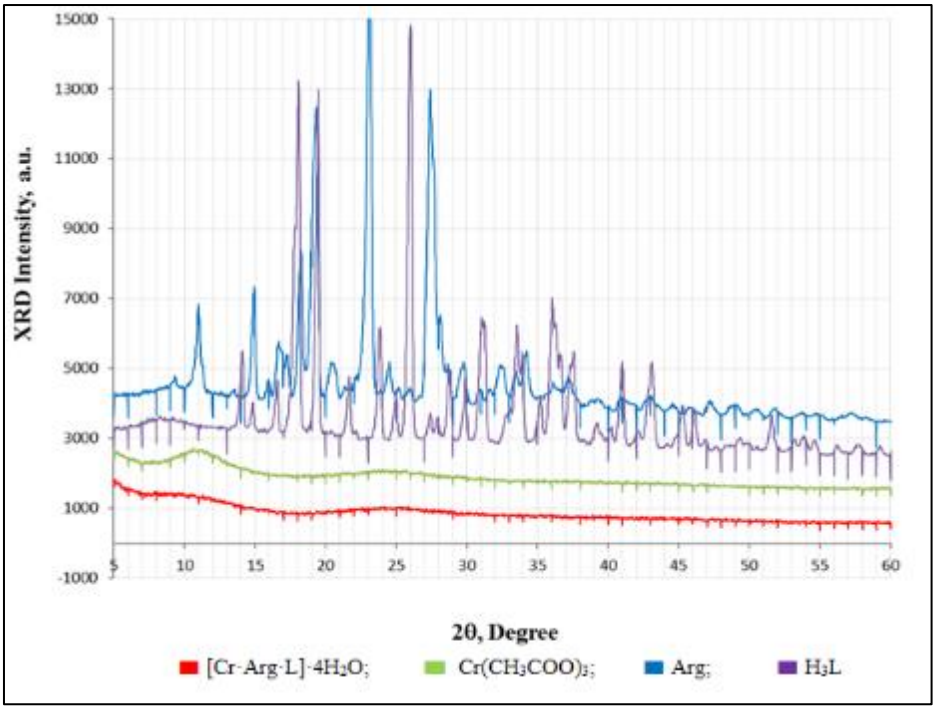

Figure 3 Diffractogram of the compound [Cr·Arg·L]·4H_2O

Based on the conducted diffractographic study, as is seen from the figures, these compounds [CoArgHL]·4H_2O (1) and Cr·Arg·L·4H_2O (3) are obtained in amorphous state. They don't contain initial substances (cobalt acetate, chromium acetate, arginine and citric acid in the form of mixture), which are characterized by diffraction maximums and intensities peculiar to them.

As for the compound [Cu(Arg)HL]·6H_2O (2), it also has been obtained in crystalline state and, according to Figure 2, it doesn't contain reacting components in the form of mixture; reacting substances are also in crystalline state with diffraction maximums and intensities peculiar to them.

Humidity has been measured using the analyzer AXIS ADGS50, and it varies within limits of 0.46-1.97 (Table 1).

In order to determine dissociation degree and dissociation constant of chelate citrates, a conductometric study has been carried out using the device pH and Conductivity Sensor LE703. For this purpose, the diluted solutions with concentrations within limits of 0.001 N-0.00002 N have been prepared for these compounds. Experiment has been conducted in thermostat at 25°C. Experimental results are given in Table 1. R^2 – determination coefficient (regression assessment indicator), which indicates how close are the experimental data to respective functions of the graph, is quite high and varies within limits of 0.73-0.83. As for the value of dissociation constant, it doesn't depend on solution dilution rate and is a constant value. As is seen from Table, it is low enough and varies within limits of 4.60 – 3.57 (Table 1).

In order to study an effect of premixes prepared on the basis of synthesized compounds on rabbits' physiological state, prior to their slaughter the blood samples have been taken for study of some hematological indicators. Analysis results are given in Table 2. Premix of the first test group was supplemented with a minimal dose of a mixture prepared on the basis of cobalt, chromium and copper chelates, that of the second test group – with maximal dose, while premixes available at poultry factory have been used for the control group.

Blood hemoglobin content points at the intense work of blood-forming organs, as well as the state of a liver, level of supply with nutritional substances, especially high-value proteins and microelements, the course of oxidation-reduction processes running in organism etc. In our case, hemoglobin content in blood of animals of all three groups is satisfactory, since according to the norm, this index varies from 105 to 125 g/l in rabbit blood (Table 2).

Table 2 Some hematological indicators of rabbits' blood

#	Indicators	Groups		
		I	II	III
		I Experimental	II Experimental	experimental Control
1	Hemoglobin HGB	110	112	118
2	Erythrocytes RBC	$4.6 \cdot 10^{12}$	$5.0 \cdot 10^{12}$	$4.7 \cdot 10^{12}$
3	Color index	0.83	0.89	0.83
4	Platelets PTL	120	245	250
5	Leukocytes WBC	$5.5 \cdot 10^{9}$	$5.0 \cdot 10^{9}$	$5.5 \cdot 10^{9}$
6	Neutrophils NEUT: Segmental nucleus	40	2	6
7	Eosinophils EOS	5	1	4
8	Monocytes MON	5	4	4
9	Lymphocytes LYM	44	46	46
10	ESRErythrocyte sedimentation rate ESR	2	2	2

The red blood count is satisfactory as well. In the norm, this index is within a range of $4,5\text{-}7,5 \cdot 10^{12}$ l.

Leucocyte count (white blood count), which perform organism protection function and promote tissue multiplication and regeneration, as well as red blood count, demonstrate metabolism processes running in organism.

In the norm, white blood count for rabbits has to vary within a range of $6,5\text{-}9,5 \cdot 10^{9}$ l. According to our test results, this index is a little lower in blood of test live-stock and varies within $5,5 \cdot 10^{9}$ – for the first group, $5,0 \cdot 10^{9}$ – for the second one, and $5,5 \cdot 10^{9}$ – for the third one.

Rod nuclear cell count is also low for the first and second test group, while in the control one it is close to the norm (5-9%).

Segmentated cell count is within the norm (33-39%) in blood of control group animals only, while in the first and second groups it is slightly higher.

As for count of eosinophils (1-3%, according to the norm), it is slightly higher among animals of the first and control groups.

Count of monocytes is also within the norm (1-4%) in the second test and control groups, while in the first test group it is a little bit higher.

As for erythrocyte deposition rate (1-3%, according to the norm), it is the same for blood of animals of all groups, while lymphocyte count is a little behind the norm.

4. Conclusions

Based on the carried-out experiments, one may draw the following conclusions:

- Synthesis conditions have been established and arginine-containing cobalt, copper and chromium citrates have been synthesized;
- The synthesized compounds have been studied using a number of physical and chemical research methods (melting, solubility, humidity, x-ray diffraction, trace element analysis);
- When studying the effect of premixes prepared on the basis of synthesized chelate compounds on rabbits' physiological state, it has been established that an improvement of rabbits' blood biochemical indicators took

place, which is a consequence of improvement of animals' physiological state, inhibition of food mass passage through gastrointestinal tract (prolongation effect) and respectively, increase of food digestibility, decrease of toxicity, enhancement of immune system stability and reduction of food intake, when using microelements as chelate compounds.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed. The authors of the manuscript no conflicts of interest have

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